

Page 50, paragraph beginning line 21:

A further object of this invention provides for cells, tissue, plants, pollen derived from said transformation of the mutant *Synechocystis pds* gene and the *ahas* genes into untransformed plant cells, using the processes mentioned above. Alternatively, mutant forms of *pds* genes with mutation(s) at position(s) similar to the *Synechocystis* gene can be obtained for any given crop species, and used further for genetic transformation. *Synechocystis* mutant *pds* gene(s) resistant to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide and the mutant AHAS gene comprising the *ahas* small subunit and the *ahas* large subunit identified in these processes can be, respectively, introduced directly into crops for engineering 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide resistance via chloroplast-mediated transformation and imidazolinone resistance. The genes can also be used for generating resistance to other *pds* or AHAS inhibiting herbicides.

IN THE CLAIMS

Please cancel claims 1-10.

-- 11 (New). An isolated and purified polynucleotide consisting of a mutant *pds* gene from a cyanobacterium, wherein said mutant *pds* gene encodes resistance to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 12 (New). An isolated and purified polynucleotide according to claim 11, wherein said cyanobacterium is selected from the group consisting of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120. --

-- 13 (New). An isolate and purified polynucleotide according to claim 11, wherein said mutant *pds* gene has a sequence comprising SEQUENCE ID NO. 3. --

-- 14 (New). An isolated and purified polynucleotide according to claim 11, wherein said mutant *pds* gene encodes cross-resistance to a group consisting of (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyridinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl]. --

-- 15 (New). A replicable expression vector comprising the polynucleotide of Claim 11. --

-- 16 (New). A nuclear genome comprising the replicable expression vector of claim 15. --

-- 17 (New). A plastome comprising the replicable expression vector of claim 15. --

-- 18 (New). A transgenic plant produced from transformation with the replicable expression vector according to Claim 15. --

-- 19 (New). A transgenic plant according to claim 18, wherein said transgenic plant exhibits resistance to herbicides

selected from the group consisting of 4'-fluoro-6-
[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, (2E)-2-
[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-
butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-
6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-
benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-
9,10-dihydro-9,10-dioxo-1-anthracenyl]. --

-- 20 (New). Progeny derived from the transgenic plant according to claim 18. --

-- 21 (New). A selectable marker for transformation comprising a mutant cyanobacterial *pds* gene containing the polynucleotide of Claim 11. --

-- 22 (New). A process for selection for new traits such as herbicide resistance, comprising the use of a mutant cyanobacterial *pds* gene of Claim 11, coupled with the selection on PDS inhibitors. --

-- 23 (New). A process for selection for new traits according to claim 22, wherein said PDS inhibitor is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 24 (New). An isolated and purified polynucleotide consisting of a mutant *pds* gene, wherein said mutant *pds* gene has a base pair mutation change of guanine to adenine at position 642 of said mutant *pds* gene. --

-- 25 (New). An isolated and purified polynucleotide according to claim 24, wherein said cyanobacterium is selected from the group consisting of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120. --

-- 26 (New). An isolate and purified polynucleotide according to claim 24, wherein said mutant *pds* gene has a sequence comprising SEQUENCE ID NO. 3. --

-- 27 (New). An isolated and purified polynucleotide according to claim 24, wherein said mutant *pds* gene encodes cross-resistance to a group consisting of (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl]. --

-- 28 (New). A replicable expression vector comprising the polynucleotide sequence of Claim 24. --

-- 29 (New). A nuclear genome comprising the replicable expression vector of claim 28. --

-- 30 (New). A plastome comprising the replicable expression vector of claim 28. --

-- 31 (New). A transgenic plant produced from transformation with the replicable expression vector according to Claim 28. --

-- 32 (New). A transgenic plant according to claim 31, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-

9,10-dihydro-9,10-dioxo-1-anthracenyl. --

-- 33 (New). Progeny derived from the transgenic plant according to claim 31. --

-- 34 (New). A selectable marker for transformation comprising a mutant cyanobacterial *pds* gene containing the polynucleotide of Claim 24. --

-- 35 (New). A process for selection for new traits such as herbicide resistance, comprising the use of a mutant cyanobacterial *pds* gene of Claim 24, coupled with the selection on PDS inhibitors. --

-- 36 (New). A process for selection for new traits according to claim 35, wherein said PDS inhibitor is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 37 (New). A selectable marker for transformation comprising a polynucleotide that confers resistance to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 38 (New). An isolated and purified polynucleotide, encoding an AHAS large subunit gene from a cyanobacterium. --

-- 39 (New). An isolated and purified polynucleotide according to claim 38, wherein the cyanobacterium is extracted from *Synechocystis* PCC 6803. --

-- 40 (New). An isolated and purified polynucleotide according to claim 38, wherein said AHAS large subunit gene confers resistance to a herbicide selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcaboxamides. --

-- 41 (New). An isolated and purified polynucleotide according to claim 38, wherein said polynucleotide consists of a sequence comprising SEQUENCE ID NO. 6. --

-- 42 (New). A replicable expression vector comprising the polynucleotide of claim 38. --

-- 43 (New). A nuclear genome comprising the replicable expression vector of claim 42. --

-- 44 (New). A plastome comprising the replicable expression vector of claim 42. --

-- 45 (New). A transgenic plant produced from transformation with the replicable expression vector according to Claim 42. --

-- 46 (New). Progeny derived from the transgenic plant according to claim 45. --

-- 47 (New). A transgenic plant according to claim 45, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcaboxamides. --

-- 48 (New). A replicable expression vector according to claim 42, wherein said replicable expression vector is a construct for nuclear genome transformation comprising an *Arabidopsis* AHAS large subunit promoter and transit sequence, the *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence. --

-- 49 (New). A selectable marker for transformation

comprising a cyanobacterial AHAS subunit containing the polynucleotide of Claim 38. --

-- 50 (New). A process for selection for new traits such as herbicide resistance comprising the use of a cyanobacterial AHAS subunit of Claim 38, coupled with the selection on an imidazolinone. --

-- 51 (New). A process for selection according to claim 50, wherein said imidazolinone is imazethapyr. --

-- 52 (New). An isolated and purified polynucleotide encoding an AHAS small subunit gene from a cyanobacterium. --

-- 53 (New). An isolated and purified polynucleotide according to claim 52, wherein the cyanobacterium *Synechocystis* PCC 6803. --

-- 54 (New). An isolated and purified polynucleotide according to claim 52, wherein said AHAS small subunit gene confers resistance to a herbicide selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides. --

-- 55 (New). An isolated and purified polynucleotide according to claim 52, wherein said polynucleotide consists of a sequence comprising SEQUENCE ID NO. 17. --

-- 56 (New). A replicable expression vector comprising the polynucleotide of claim 52. --

-- 57 (New). A nuclear genome comprising the replicable expression vector of claim 56. --

-- 58 (New). A plastome comprising the replicable expression vector of claim 56. --

-- 59 (New). A transgenic plant produced from transformation with the replicable expression vector according to Claim 56. --

-- 60 (New). Progeny derived from the transgenic plant according to claim 59. --

-- 61 (New). A transgenic plant according to claim 59, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides. --

-- 62 (New). A replicable expression vector according to claim 56, wherein said replicable expression vector is a construct for nuclear genome transformation comprising an *Arabidopsis* AHAS large subunit promoter and transit sequence, the *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence. --

-- 63 (New). A selectable marker for transformation, comprising a cyanobacterial AHAS subunit containing the polynucleotide of Claim 52. --

-- 64 (New). A process for selection for new traits such as herbicide resistance comprising the use of a cyanobacterial AHAS subunit of Claim 52, coupled with the selection on an imidazolinone. --

-- 65 (New). A process for selection according to claim 64, wherein said imidazolinone is imazethapyr. --

-- 66 (New). A rapid plate assay screening method designed to identify inhibitors of specific metabolic pathways, common to photoautotrophic cyanobacteria and higher plants, comprising the steps of:

inoculating cyanobacteria into a simple growth medium;
adding test compounds to the growth medium; and
noting which test compounds inhibit the growth of the cyanobacterium within one to three days. --

-- 67 (New). The rapid plate assay screening method according to claim 66, wherein the cyanobacteria are selected from the group consisting of *Synechocystis* PCC 6803, *Anabaena* PCC 7120, and a mixture of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120. --

-- 68 (New). A rapid plate assay screening method according to claim 66, wherein the growth medium is 2x BG-11. --

-- 69 (New). The rapid plate assay screening method according to claim 66, wherein at least one of the test compounds is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 70 (New). A method to isolate and select mutants resistant to herbicides comprising:
treating algae cell cultures with a chemical that kills the algae cell cultures at a high killing rate;
quenching the chemical reaction with the addition of a second chemical;
plating the surviving algae cell cultures on a solid medium containing a concentration of a herbicide; and
collecting surviving algae cell cultures. --

-- 71 (New). The method according to claim 70, wherein the chemical for creating a chemical reaction is ethyl methanesulfonate. --

-- 72 (New). The method according to claim 70, wherein the second chemical is sodium thiosulfate. --

-- 73 (New). The method according to claim 70, wherein the herbicide is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. --

-- 74 (New). The method according to claim 73, wherein the concentration of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide is 1uM - 5uM. --

-- 75 (New). A method to isolate and select mutants resistant to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide comprising:
treating algae cell cultures with ethyl methanesulfonate, which kills the algae cell cultures at a high killing rate;
quenching the chemical reaction with the addition of a sodium thiosulfate;
plating the surviving algae cell cultures on a solid medium containing 1uM - 5uM of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide;
collecting surviving algae cell cultures; and
selecting a fragment from herbicide resistant cell lines by using two primers, cgaattccctggtagcatttaatacaattggc and cgcataagctttgcagatggagacggtttgggc. --

-- 76 (New). A method for improved genetic transformation of cyanobacteria comprising the steps of:

- a) placing competent cyanobacteria into transforming medium in well plates;
- b) adding a transforming nucleotide species to the transforming medium;
- c) replica plating the cyanobacterium, at least two different time intervals on selection plates containing at least one selection agent. --

-- 77 (New). The method according to claim 76, wherein the cyanobacteria are *Synechocystis*. --

- 78 (New). A method for transforming plastomes with cyanobacterial nucleic acid fragments encoding herbicidal resistance comprising the steps of:
 - a) isolating a cyanobacterial nucleic acid fragment encoding herbicide resistance;
 - b) incorporating the nucleic acid fragment of step (a) into an expression vector;
 - c) incorporating the expression vector of step (b) into a plasmid;
 - d) cutting leaves from a plant and placing them abaxial side down; and
 - e) bombarding the leaves with the plasmid of step (c). --

-- 79 (New). A method for transforming plastomes according to claim 78, wherein the cyanobacterial nucleic acid fragments are derived from a gene encoding a cyanobacterial enzyme selected from the group consisting of a mutant pds enzyme encoding resistance to 4'-fluoro-6- [(alpha, alpha, alpha, -trifluoro-m-tolyl) oxy] - picolinamide, a large AHAS subunit and a small AHAS subunit. --

-- 80 (New). The method for transforming plastomes according to claim 78, wherein the expression vector comprises an *Arabidopsis* AHAS large subunit promoter and transit sequence, a *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence. --

-- 81 (New). The method for transforming plastomes according to claim 78, wherein the plastomes are chloroplasts. --

-- 82 (New). The method for transforming plastomes according to claim 78, wherein the plasmids are selected from the group consisting of p116 I, p116 II, p12delta NI, and p12delta NII. --

- 83 (New). A method for target site gene identification in an organism for which a complete genomic sequence is available, comprising the steps of:
 - a) identifying a lead compound which affects the activity of at least one gene of the organism;
 - b) generating a cell line from the organism which is resistant to the lead compound of step (a);
 - c) isolating genomic DNA fragments from the resistant cell line of step (b);
 - d) preparing primer pairs for PCR amplification comprising overlapping DNA fragments from the entire genomic sequence of the organism;
 - e) amplifying the DNA fragments from the resistant cell line of step (c) by PCR using the primer of step (d) to form amplified DNA fragments from the resistant cell line;
 - f) transforming competent cells from the organism with the amplified DNA fragments to obtain transformed cells;
 - g) screening the transformed cells for resistance to the lead compound to obtain resistant transformed cells; and
 - h) matching the resistant transformed cells to the primers that amplified the DNA used to transform the resistant transformed cells; and